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(88) Date of publication of the international search report:
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR ASSAYING REPLICATION OF HBV AND TESTING SUSCEPTIBILITY TO DRUGS

(57) Abstract: A method for measuring the replication capacity of HBV, e.g. HBV present in a biological sample, possibly in the presence of a pharmaceutical product, in particular an antiviral agent, the method comprising: (a) possibly extracting nucleic acids contained in the biological sample; (b) PCR amplifying HBV nucleic acids using at least two primer pairs selected so as to obtain at least two different amplified HBV genomic fragments which upon assembly represent a linear continuous DNA sequence transcriptable in pgRNA ; (c) cloning the fragments obtained under (b) into a vector under the control of an heterologous promoter, so producing a vector containing a linear continuous DNA sequence transcriptable in pgRNA under control of said promoter; (d) transfecting or transducing susceptible cells with the vector; (e) culturing the transfected or transduced cells in conditions allowing synthesis of HBV pgRNA from the cloned HBV DNA; (f) possibly treating the cultured cells with the pharmaceutical product, in particular antiviral agent; and (g) determining the replication capacity of the HBV, possibly incidence of the pharmaceutical product, preferably antiviral agent, on viral gene expression and/or viral replication.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/12398

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, MEDLINE, PAJ, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHORIES MARCUS ET AL: "Isolation, characterization and biological significance of hepatitis B virus mutants from serum of a patient with immunologically negative HBV infection." JOURNAL OF HEPATOLOGY, vol. 33, no. 5, November 2000 (2000-11), pages 799-811, XP002246280 ISSN: 0168-8278 the whole document ----- -/--	1-34

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 April 2004	04. 10. 2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Botz, J

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/12398

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ULRICH P P ET AL: "A PRECORE-DEFECTIVE MUTANT OF HEPATITIS B VIRUS ASSOCIATED WITH E ANTIGEN-NEGATIVE CHRONIC LIVER DISEASE" JOURNAL OF MEDICAL VIROLOGY, vol. 32, no. 2, 1990, pages 109-118, XP008019147 ISSN: 0146-6615 the whole document	1-34
X	GUNTHER STEPHAN ET AL: "Amplification of full-length hepatitis B virus genomes from samples from patients with low levels of viremia: Frequency and functional consequences of PCR-introduced mutations." JOURNAL OF CLINICAL MICROBIOLOGY, vol. 36, no. 2, February 1998 (1998-02), pages 531-538, XP002246281 ISSN: 0095-1137 the whole document	1-34
Y	DELANEY W E 4TH ET AL: "Use of the hepatitis B virus recombinant baculovirus-HepG2 system to study the effects of (-)-beta-2',3'-dideoxy-3'-thiacytidine on replication of hepatitis B virus and accumulation of covalently closed circular DNA." ANTIMICROBIAL AGENTS AND CHEMOTHERAPY. UNITED STATES AUG 1999, vol. 43, no. 8, August 1999 (1999-08), pages 2017-2026, XP002246282 ISSN: 0066-4804 the whole document	1-34
Y	DATABASE EBI [Online] 6 October 1999 (1999-10-06), FRANK, B.L., ET AL.: "Sequence 5 from patent US 5856459" XP002246286 accession no. EMBL Database accession no. AR027807 the whole document & US 5 856 459 A (HYBRIDON, INC.) 5 January 1999 (1999-01-05) table 1	1-34

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/12398

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE GENBANK [Online] 23 March 2001 (2001-03-23), SUGAUCHI, F. ET AL.: "Hepatitis B virus DNA, complete genome, serotype: ayw" XP002246287 retrieved from GI:13365548 accession no. NCBI Database accession no. AB048704 cited in the application the whole document	1-34
Y	KRUINING JOHANNES ET AL: "Antiviral agents in hepatitis B virus transfected cell lines: Inhibitory and cytotoxic effect related to time of treatment." JOURNAL OF HEPATOLOGY, vol. 22, no. 3, 1995, pages 263-267, XP002246283 ISSN: 0168-8278 the whole document	1-34
A	DELMAS JULIEN ET AL: "Inhibitory effect of adefovir on viral DNA synthesis and covalently closed circular DNA formation in duck hepatitis B virus-infected hepatocytes in vivo and in vitro." ANTIMICROBIAL AGENTS AND CHEMOTHERAPY. UNITED STATES FEB 2002, vol. 46, no. 2, February 2002 (2002-02), pages 425-433, XP002246284 ISSN: 0066-4804 the whole document	
A	BECK J ET AL: "Reconstitution of a functional duck hepatitis B virus replication initiation complex from separate reverse transcriptase domains expressed in Escherichia coli." JOURNAL OF VIROLOGY. UNITED STATES AUG 2001, vol. 75, no. 16, August 2001 (2001-08), pages 7410-7419, XP002246285 ISSN: 0022-538X the whole document	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/12398

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1,2,5-8,11-13,15,17; (incompletely searched)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-34 (partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1,2,5-8,11-13,15,17; (incompletely searched)

Present claims 1 relates to an extremely large number of possible products. In fact, the claims contain so many variables, that a lack of clarity and conciseness within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Claim 1 (c.) uses the term "a linear continous DNA sequence transcriptable in pgRNA". This term is unclear and not sufficiently defined. Consequently, the search has been carried out for those parts of the application which do appear to be clear and concise. In the present case the method was searched for a full-length- HBV-DNA. Claim 2: the term "an about 1 genome unit" has been searched in the meaning of "one full-length genome of HBV". A "sub-genomic fragment..." has been searched as part of the genome comprising the pre-C gene and including the poly-A attachment site. Present claims 5 - 8 relate to extremely large number of possible products. These claims lack clarity and conciseness within the meaning of Article 6 PCT. A meaningful search is impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear and concise. In claim 5, the first primer pair and the second primer pair remain undefined in terms of their sequence. As a consequence of that the forward primer A is also undefined in claim 6. In addition, claim 7 is formulated as an effect to be achieved ("a restricition site which is not present in the HBV-genome"). Claims 5 - 8 have only been searched with respect to their concrete embodiments, namely the SEQ. ID. Nos. provided in the underlying application. In the same respect claims 11-13,15 are unclear and claim 17 is formulated as an effect to be achieved. These claims have only been searched with respect to their concrete embodiments, namely the SEQ. ID. Nos. provided in the underlying application.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-34 (partially)

Invention Number 1:
The Polynucleotide with the Sequence Identity Number 1, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

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2. claims: 1-34 (partially)

Invention Number 2:
The Polynucleotide with the Sequence Identity Number 2, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

---

3. claims: 1-34 (partially)

Invention Number 3:
The Polynucleotide with the Sequence Identity Number 3, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

---

4. claims: 1-34 (partially)

Invention Number 4:
The Polynucleotide with the Sequence Identity Number 4, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

---

5. claims: 1-34 (partially)

Invention Number 5:
The Polynucleotide with the Sequence Identity Number 5, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

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6. claims: 1-34 (partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Invention Number 6:
The Polynucleotide with the Sequence Identity Number 6, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

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7. claims: 1-34 (partially)

Invention Number 7:
The Polynucleotide with the Sequence Identity Number 7, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

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8. claims: 1-34 (partially)

Invention Number 8:
The Polynucleotide with the Sequence Identity Number 8, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

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9. claims: 1-34 (partially)

Invention Number 9:
The Polynucleotide with the Sequence Identity Number 9, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

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10. claims: 1-34 (partially)

Invention Number 10:
The Polynucleotide with the Sequence Identity Number 10, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

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11. claims: 1-34 (partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Invention Number 11:
The Polynucleotide with the Sequence Identity Number 11, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

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12. claims: 1-34 (partially)

Invention Number 12:
The Polynucleotide with the Sequence Identity Number 12, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a Kit for HBV amplification.

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13. claims: 1-34 (partially)

Invention Number 13:
The Polynucleotide with the Sequence Identity Number 13, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

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14. claims: 1-34 (partially)

Invention Number 14:
The Polynucleotide with the Sequence Identity Number 14, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

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15. claims: 1-34 (partially)

Invention Number 15:
The Polynucleotide with the Sequence Identity Number 15, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

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16. claims: 1-34 (partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Invention Number 16:
The Polynucleotide with the Sequence Identity Number 16, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

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17. claims: 1-34 (partially)

Invention Number 17:
The Polynucleotide with the Sequence Identity Number 17, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

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18. claims: 1-34 (partially)

Invention Number 18:
The Polynucleotide with the Sequence Identity Number 18, its use in a method for measuring the replication capacity of HBV, it being useful as primer for HBV amplification, it being comprised in a primer pair for HBV amplification, it being comprised in a kit for HBV amplification.

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19. claim: 35 (completely)

Invention Number 19:
Vector comprising a continous HBV DNA and a promoter;

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20. claim: 36 (completely)

Invention Number 20:
A baculovirus or cell line comprising the vector of claim 35;

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 03/12398

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5856459 A	05-01-1999	AT 233318 T	15-03-2003
		AU 6125296 A	24-12-1996
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		DE 69626393 D1	03-04-2003
		DE 69626393 T2	04-12-2003
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